

U.S.S.N. 08/259,321
FILED: JUNE 10, 1994
AMENDMENT

APPENDIX : Claims as pending upon entry of this amendment

1. (four times amended) A recombinant Ca^{2+} dependent monoclonal antibody or antibody fragment including a heavy chain and a light chain, wherein the antibody or antibody fragment comprise the hypervariable regions of the monoclonal antibody produced by the hybridoma deposited with the American Type Culture Collection as ATCC No. HB 9892 which bind an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) and calcium ions, where the antibody and antibody fragment inhibit Protein C activation by thrombin-thrombomodulin, and wherein the antibody and antibody fragment are expressed in bacterial or insect cells or is humanized.

2. (twice amended) The antibody of claim 1 comprising an amino acid sequence selected from the group consisting of:

MGR LSSS FLL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT
SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10);
MDFQVQIFS F LLISASVIMS RGQILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK
SGTSPKLLIY GTSNLAGVPSRFSGSGSGT FYSLTVSSVE AEDAADYYCH
QWNSYPHTFG GGTKLEIKR (Sequence ID No. 12); [Q VTLKESGPGI LQPSQTLTLT
CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII
SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Jamino

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acids 20-139 of Sequence ID No. 10[] and [QIILTQSP AIMSASLGEE ITLTCSATSS
VTYVHWYQQK SGTSPKLLIY GTSNLAGVPSRFSGSGSGT FYSLTVSSVE
AEDAADYYCH QWNSYPHTFG GGTKLEIKR (J amino acids 23-129 of Sequence ID No.
12[]).

3. (five times amended) The antibody of claim 1 which is humanized.[biological fluid.]
5. (amended) A composition comprising the antibody of claim 1 in combination with a pharmaceutically acceptable carrier for administration to a patient.
7. (amended) The antibody of claim 1 having a detectable label directly bound to the antibody.
8. (twice amended) The antibody of claim 1 immobilized to a substrate which does not interfere with binding of the antibody to protein C in combination with calcium ions, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.
14. (four times amended) A method of making a recombinant Ca^{2+} dependent monoclonal antibody which binds an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) and calcium ions, where the antibody inhibits Protein C activation by thrombin-thrombomodulin, by expressing nucleotide molecules encoding the hypervariable region of the heavy and light chains of the monoclonal antibody expressed by the hybridoma deposited with the American Type Culture Collection as ATCC No. HB 9892 in bacteria or insect cells.
15. (twice amended) The method of claim 14 wherein the antibody comprises an

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amino acid sequence selected from the group consisting of:

MGRLLSSSFLI LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT

SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF

LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10);

MDFQVQIFS LLIASVIMS RGQILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK

SGTSPKLLIY GTSNLAGVPSRFSGSGSGT FYSLTVSSVE AEDAADYYCH

QWNSYPHTFG GGTKLEIKR (Sequence ID No. 12); [Q VTLKESGPGI LQPSQTLTLT

CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII

SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (amino

acids 20-139 of Sequence ID No. 10)] and [QILTQSP AIMSASLGEE ITLTCSATSS

VTYVHWYQQK SGTSPKLLIY GTSNLAGVPSRFSGSGSGT FYSLTVSSVE

AEDAADYYCH QWNSYPHTFG GGTKLEIKR (amino acids 23-129 of Sequence ID No.

12)].

17. (four times amended) The method of claim 14 wherein the antibody is humanized.

18. (amended) The method of claim 14 further comprising directly binding detectable label to the antibody.

19. (amended) The method of claim 14 further comprising immobilizing the antibody to a substrate which does not interfere with binding of the antibody to protein C in combination with calcium ions, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

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20. (amended) The recombinant antibody of claim 1 having coupled thereto a peptide sequence.

21. (amended) The method of claim 14 wherein the nucleotide sequence encoding the recombinant antibody is ligated to a sequence encoding a peptide and the ligated nucleotide sequence is expressed in an expression system.